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(54) Title: ROTAMASE ENZYME ACTIVITY INHIBITORS

(57) Abstract

This invention relates to the method of using specially formulated neurotrophic pipecolic acid derivative compounds having an affinity for FKBP-type immunophilins as inhibitors of the enzyme activity associated with immunophilin proteins, and particularly inhibitors of peptidyl-prolyl isomerase or rotamase enzyme activity to stimulate or promote neuronal growth or regeneration.

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ROTAMASE ENZYME ACTIVITY INHIBITORS

Related Application

This application is a continuation-in-part application of U.S. Patent Application Serial No. 08/551,026 filed October 31, 1995.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to the method of using neurotrophic FKBP inhibitor compounds having an affinity for FKBP-type immunophilins as inhibitors of the enzyme activity associated with immunophilin proteins, and particularly inhibitors of peptidyl-prolyl isomerase or rotamase enzyme activity.

2. Description of the Prior Art

The term immunophilin refers to a number of proteins that serve as receptors for the principal immunosuppressant drugs, cyclosporin A (CsA), FK506, and rapamycin. Known classes of immunophilins are cyclophilins, and FK506 binding proteins, such as FKBP. Cyclosporin A binds to cyclophilin while FK506 and rapamycin bind to FKBP. These immunophilin-drug complexes interface with a variety of intracellular signal transduction systems, especially in the immune system and the nervous system.

Immunophilins are known to have peptidyl-prolyl isomerase (PPIase) or rotamase enzyme activity. It has been determined that rotamase activity has a role in the catalyzation of the interconversion of

the cis and trans isomer of immunophilin proteins.

Immunophilins were originally discovered and studied in immune tissue. It was initially postulated by those skilled in the art that inhibition of the immunophilins rotamase activity leads to the inhibition of T-cell proliferation, thereby causing the immunosuppressive action exhibited by immunosuppressive drugs such as cyclosporin A, FK506, and rapamycin. Further study has shown that the inhibition of rotamase activity, in and of itself, is not sufficient for immunosuppressant activity. Instead immunosuppression appears to stem from the formulation of a complex of immunosuppressant drugs and immunophilins. It has been shown that the immunophilin-drug complexes interact with ternary protein targets as their mode of action. In the case of FKBP-FK506 and FKBP-CsA, the drug-immunophilin complexes bind to the enzyme calcineurin, inhibiting T-cell receptor signalling leading to T-cell proliferation. Similarly, the complex of rapamycin and FKBP interacts with the RAFT1/FRAP protein and inhibits signalling from the IL-2 receptor.

Immunophilins have been found to be present at high concentrations in the central nervous system. Immunophilins are enriched 10-50 times more in the central nervous system than in the immune system.

Within neural tissues, immunophilins appear to influence nitric oxide synthesis, neurotransmitter release, and neuronal process extension.

FK506 also augments the phosphorylation of growth-associated protein-43 (GAP43). GAP43 is involved in neuronal process extension and its phosphorylation appears to augment this activity. Accordingly, the effects of FK506 rapamycin and cyclosporin in neuronal process extension have been examined using PC12 cells. PC12 cells are a continuous line of neuronal-like cells which extend neurites when stimulated by nerve growth factor (NGF).

Surprisingly, it has been found that picomolar concentrations of an immunosuppressant such as FK506 and rapamycin stimulate neurite out growth in PC12 cells and sensory neurons, namely dorsal root ganglion cells (DRGs). In whole animal experiments, FK506 has been shown to stimulate nerve regeneration following facial nerve injury and results in functional recovery in animals with sciatic nerve lesions.

More particularly, it has been found that drugs with a high affinity for FKBP are potent rotamase inhibitors and exhibit excellent neurotrophic effects. Snyder et al., "Immunophilins and the Nervous System", *Nature Medicine*, Volume 1, No. 1, January 1995, 32-37. These findings suggest the use

of immunosuppressants in treating various peripheral neuropathies and enhancing neuronal regrowth in the central nervous system (CNS). Studies have demonstrated that neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease may occur due to the loss, or decreased availability, of a neurotrophic substance specific for a particular population of neurons affected in the disorder.

Several neurotrophic factors effecting specific neuronal populations in the central nervous system have been identified. For example, it has been hypothesized that Alzheimer's disease results from a decrease or loss of nerve growth factor (NGF). It has thus been proposed to treat SDAT patients with exogenous nerve growth factor or other neurotrophic proteins such as brain derived growth factor, glial derived growth factor, ciliary neurotrophic factor, and neurotropin-3 to increase the survival of degenerating neuronal populations.

Clinical application of these proteins in various neurological disease states is hampered by difficulties in the delivery and bioavailability of large proteins to nervous system targets. By contrast, immunosuppressant drugs with neurotrophic activity are relatively small and display excellent bioavailability and specificity. However, when administered chronically, immunosuppressants exhibit a number of potentially serious side effects

including nephrotoxicity, such as impairment of glomerular filtration and irreversible interstitial fibrosis (Kopp et al., 1991, *J. Am. Soc. Nephrol.* 1:162); neurological deficits, such as involuntary tremors, or non-specific cerebral angina such as non-localized headaches (De Groen et al., 1987, *N. Engl. J. Med.* 317:861); and vascular hypertension with complications resulting therefrom (Kahan et al., 1989 *N. Engl. J. Med.* 321: 1725).

The present invention provides non-immunosuppressive FKBP inhibitor compounds containing small molecule FKBP rotamase inhibitors which are extremely potent in augmenting neurite outgrowth, and for promoting neuronal growth, and regeneration in various neuropathological situations where neuronal repair can be facilitated including peripheral nerve damage by physical injury or disease state such as diabetes, physical damage to the central nervous system (spinal cord and brain), brain damage associated with stroke, and for the treatment of neurological disorders relating to neurodegeneration, including Parkinson's disease and Alzheimer's disease.

SUMMARY OF THE INVENTION

This invention relates to the method of using neurotrophic FKBP inhibitor compounds having an affinity for FKBP-type immunophilins as inhibitors of the enzyme activity associated with immunophilin

proteins, and particularly inhibitors of peptidyl-prolyl isomerase or rotamase enzyme activity.

A preferred embodiment of this invention is a method of treating a neurological activity in an animal, comprising: administering to an animal an effective amount of a FKBP inhibitor having an affinity for FKBP-type immunophilins to stimulate growth of damaged peripheral nerves or to promote neuronal regeneration, wherein the FKBP-type immunophilin exhibits rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

Another preferred embodiment of this invention is a method of treating a neurological disorder in an animal, comprising: administering to an animal an effective amount of a FKBP inhibitor having an affinity for FKBP-type immunophilins in combination with an effective amount of a neurotrophic factor selected from the group consisting of neurotrophic growth factor, brain derived growth factor, glial derived growth factor, cilial neurotrophic factor, and neurotropin-3, to stimulate growth of damaged peripheral nerves or to promote neuronal regeneration, wherein the FKBP-type immunophilin exhibits rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

Another preferred embodiment of this invention

is a method of stimulating growth of damaged peripheral nerves, comprising: administering to damaged peripheral nerves an effective amount of an FKBP inhibitor compound having an affinity for FKBP-type immunophilins to stimulate or promote growth of the damaged peripheral nerves, wherein the FKBP-type immunophilins exhibit rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

Another preferred embodiment of this invention is a method of stimulating growth of damaged peripheral nerves, comprising: administering to damaged peripheral nerves an effective amount of an FKBP inhibitor compound having an affinity for FKBP-type immunophilins to stimulate growth of damaged peripheral nerves, wherein the FKBP-type immunophilin exhibit rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

Another preferred embodiment of this invention is a method for promoting neuronal regeneration and growth in animals, comprising: administering to an animal an effective amount of an FKBP inhibitor compound having an affinity for FKBP-type immunophilins to promote neuronal regeneration, wherein the FKBP-type immunophilins exhibit rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

Yet another preferred embodiment of this invention is a method for preventing neurodegeneration in an animal, comprising: administering to an animal an effective amount of an FKBP inhibitor having an affinity for FKBP-type immunophilins to prevent neurodegeneration, wherein the FKBP-type immunophilin exhibits rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a dose-response curve for Example 15. Fig. 1 shows the data from 6-10 different concentrations used to generate typical dose-response curves, from which ED_{50} values were calculated.

FIG. 2 is a dose-response curve for Example 22. Fig. 2 shows the data from 6-10 different concentrations used to generate typical dose-response curves, from which ED_{50} values were calculated.

FIG. 3 is a representative photomicrograph of a sensory neuron responding to trophic effects of the drugs. Fig. 3 shows the dose-response for Example 21 in cultured sensory neurons.

FIG. 4 is a representative photomicrograph of a sensory neuron responding to trophic effects of the drugs. Fig. 4 shows the effect of 300 pM of Example 15 on neurite outgrowth in cultured sensory neurons.

FIG. 5 is a representative photomicrograph of a sensory neuron responding to trophic effects of the drugs. Fig. 5 shows the effect of 1 nM of Example 22 on neurite outgrowth in cultured sensory neurons.

DETAILED DESCRIPTION OF THE INVENTION

The novel neurotrophic FKBP inhibitor compounds of this invention have an affinity for the FK506 binding proteins such as FKBP-12. When the neurotrophic compounds of the invention are bound to FKBP, they have been found to inhibit the prolyl-peptidyl cis-trans isomerase activity, or rotamase activity of the binding protein and unexpectedly stimulate neurite growth.

The compounds of the present invention can be used in the form of salts derived from inorganic or organic acids and bases. Included among such acid salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemissulfate heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Base salts

include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salt with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups can be quarternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates such as dimethyl, diethyl, dibutyl and diamyl sulfates; long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides; aralkyl halides like benzyl and phenethyl bromides; and others. Water or oil-soluble or dispersible products are thereby obtained.

The neurotrophic compounds of this invention can be periodically administered to a patient undergoing treatment for neurological disorders or for other reasons in which it is desirable to stimulate neuronal regeneration and growth, such as in various peripheral neuropathic and neurological disorders relating to neurodegeneration. The compounds of this invention can also be administered to mammals other than humans for treatment of various mammalian neurological disorders.

The novel compounds of the present invention are potent inhibitors of rotamase activity and

possess an excellent degree of neurotrophic activity. This activity is useful in the stimulation of damaged neurons, the promotion of neuronal regeneration, the prevention of neurodegeneration, and in the treatment of several neurological disorders known to be associated with neuronal degeneration and peripheral neuropathies. The neurological disorders that may be treated include but are not limited to: trigeminal neuralgia, glossopharyngeal neuralgia, Bell's Palsy, myasthenia gravis, muscular dystrophy, progressive muscular atrophy, progressive bulbar inherited muscular atrophy, herniated, ruptured or prolapsed invertabrae disk syndromes, cervical spondylosis, plexus disorders, thoracic outlet destruction syndromes, peripheral neuropathic such as those caused by lead, dapsone, ticks, porphyria, or Gullain-Barré syndrome, Alzheimer's disease, and Parkinson's disease.

For these purposes the compounds of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intraperitoneally, intrathecally,

intraventricularly, intrasternal and intracranial injection or infusion techniques.

To be effective therapeutically as central nervous system targets, the immunophilin-drug complex should readily penetrate the blood-brain barrier when peripherally administered. Compounds of this invention which cannot penetrate the blood-brain barrier can be effectively administered by an intraventricular route.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques know in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid and its glyceride derivatives find use in the

preparation of injectables, olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant.

The compounds may be administered orally in the form of capsules or tablets, for example, or as an aqueous suspension or solution. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The compounds of this invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The compounds of this invention may also be administered optically, especially when the

conditions addressed for treatment involve areas or organs readily accessible by topical application, including neurological disorders of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas.

For ophthalmic use, the compounds can be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions is isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively for the ophthalmic uses the compounds may be formulated in an ointment such as petrolatum.

For application topically to the skin, the compounds can be formulated in a suitable ointment containing the compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the compounds can be formulated in a suitable lotion or cream containing the active compound suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Topical application for the lower intestinal tract an be effected in a rectal suppository formulation (see above) or in a suitable enema formulation.

Dosage levels on the order of about 0.1 mg to about 10,000 mg of the active ingredient compound are useful in the treatment of the above conditions, with preferred levels of about 0.1 mg to about 1,000 mg. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

It is understood, however, that a specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated and form of administration.

The compounds can be administered with other neurotrophic agents such as neurotrophic growth factor (NGF), glial derived growth factor, brain derived growth factor, ciliary neurotrophic factor, and neurotropin-3. The dosage level of other neurotrophic drugs will depend upon the factors previously stated and the neurotrophic effectiveness of the drug combination.

Methods and Procedures

Nerve Extension Elicited in Chick Dorsal Root Ganglia by Non-Immunosuppressive Ligands of Immunophilins

In previous studies, it has been observed that neurotrophic effects of immunosuppressant drugs in explants of rat dorsal root ganglia with significant augmentation in nerve outgrowth has occurred with FK506 concentrations as low as 1 picomolar (Lyons et. al., 1994). In the rat ganglia neurotrophic effects were observed with FK506 even in the absence of NGF. In the present work explants of chick dorsal root ganglia have been used, which are easier to employ in studies of nerve outgrowth. In the absence of added NGF, we have observed minimal effects of immunophilin ligand drugs. The chick cells are more sensitive to NGF than PC-12 cells so that we employ 0.1 ng/ml NGF to produce minimal neurite outgrowth and to demonstrate neurotrophic actions of immunophilin ligands (Fig. 5).

The maximal increase in the number of processes, their length and branching is quite similar at maximally effective contractions of the immunophilin ligands and of NGF (100 ng/ml). With progressively increasing concentrations of the various drugs, one observes a larger number of processes, more extensive branching and a greater length of individual processes.

We evaluated the potencies of drugs in binding to FKBP-12 by examining inhibition of peptidyl prolyl-isomerase activity and inhibition of ^{3}H-FK506 binding to recombinant FKBP-12 (Table 1). There is a striking parallel between their potencies in stimulating neurite outgrowth and inhibiting rotamase activity.

The very close correlation between the potencies of drugs in binding to immunophilins, inhibiting their rotamase activity and stimulating neurite outgrowth implies that inhibition of rotamase activity is responsible for neurotrophic effects of the drugs. The extraordinarily high potency of the drugs in stimulating neurite outgrowth and in binding to immunophilins makes it most unlikely that any other target could account for the neurotrophic effects.

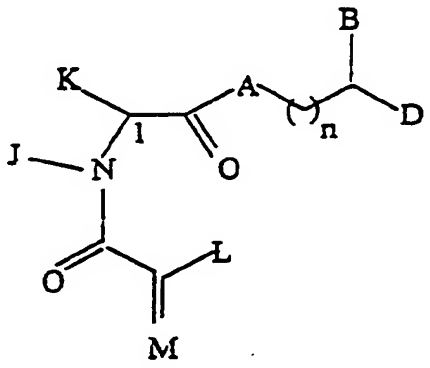
Because of the extraordinary potency of the drugs and the close correlation between rotamase inhibition and neurotrophic actions, we conclude that rotamase inhibition is likely involved in neurotrophic effects. A number of proteins have been reported as substrates for the rotamase activity of immunophilins including collagen (Steinmann et. al., 1991) and transferring (Lodish and King, 1991). Recently highly purified preparations of ryanodine receptor and the IP-3 receptor, prominent intracellular calcium channels,

have been reported to exist in a complex with FKBP-12. Dissociation of FKBP-12 from these complexes causes the calcium channels to become "leaky" (Cameron et. al., 1995). Calcium fluxes are involved in neurite extension so that the IP-3 receptor and the ryanodine receptor might be involved in the neurotrophic effects of drugs. Since the drugs bind to the same site on FKBP-12 as the IP-3 receptor or the ryanodine receptor, one would have to postulate that the drugs displace the channels from FKBP-12. No interaction between these calcium channels in cyclophilin has been reported so that this model would not explain the neurotrophic actions of cyclosporin A.

The neurotrophic actions of the drugs studied here are exerted at extremely low concentrations indicating potencies comparable to those of neurotrophic proteins such as brain derived growth factor, neurotropin-3 and neurotrophic growth factor.

The following examples are illustrative of preferred embodiments of the invention and are not to be construed as limiting the invention thereto. All polymer molecular weights are mean average molecular weights. All percentages are based on the percent by weight of the final delivery system or formulation prepared unless otherwise indicated and all totals equal 100% by weight.

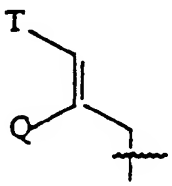
Illustrative generic FKBP inhibitor compounds which can be used for the purposes of this invention include:



and pharmaceutically acceptable salts thereof,

wherein A is CH_2, O, NH, or N-(C_1-C_4 alkyl);

wherein B and D are independently Ar, (C5-C7)-cycloalkyl substituted (C1-C6)-straight or branched alkyl or alkenyl, (C5-C7)-cycloalkenyl substituted (C1-C6)-straight or branched alkyl or alkenyl, or Ar substituted (C1-C6)-straight or branched alkyl or alkenyl, wherein in each case, one or two carbon atoms of the straight or branched alkyl or alkenyl groups may be substituted with 1-2 heteroatoms selected from the group consisting of oxygen, sulfur, SO and SO_2 in chemically reasonable substitution patterns, or



wherein Q is hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl;

wherein T is Ar or substituted 5-7 membered cycloalkyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C1-C4)-alkyl or O-(C1-C4)-alkenyl and carbonyl;

wherein Ar is selected from the group consisting of 1-napthyl, 2-napthyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl and phenyl, monocyclic and bicyclic heterocyclic ring systems with individual ring sizes being 5 or 6 which may contain in either or both rings a total of 1-4 heteroatoms independently selected from oxygen, nitrogen and sulfur; wherein Ar may contain one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, hydroxymethyl, nitro, CF3, trifluoromethoxy, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, O-benzyl, O-phenyl, amino, 1,2-methylenedioxy, carbonyl and phenyl;

wherein L is either hydrogen or U; M is either

oxygen or CH-U, provided that if L is hydrogen, then M is CH-U, or if M is oxygen then L is U;

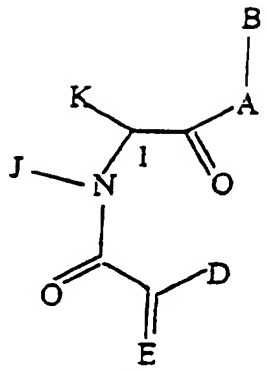
wherein U is hydrogen, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or branched alkyl or (C1-C4)-straight or branched alkenyl, [(C1-C4)-alkyl or (C1-C4)-alkenyl]-Ar or Ar (Ar as described above);

wherein J is hydrogen or C1 or C2 alkyl or benzyl; K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylethyl; or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain an oxygen (O), sulfur (S), SO or SO_2 substituted therein; and

wherein n is 0-3.

The stereochemistry at position 1 (Formula I) is (R) or (S), with (S) preferred. The stereochemistry at position 2 is (R) or (S).

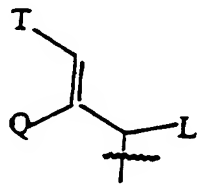
Illustrative preferred FKBP inhibitor compounds which can be used for the purposes of this invention are described in U.S. Patent No. 5,330,993, the contents of which is incorporated herein by reference. Exemplary compounds include those having the formula:



and pharmaceutically acceptable salts thereof,

wherein A is O, NH, or N-(C1-C4 alkyl);

wherein B is hydrogen, CHL-Ar, (C1-C6)-straight or branched alkyl, (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl or Ar substituted (C1-C6)-alkyl or alkenyl, or



wherein L and Q are independently hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl;

wherein T is Ar or substituted cyclohexyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C1-C4)-alkyl or O-(C1-C4)-alkenyl and carbonyl;

wherein Ar is selected from the group consisting of 1-napthyl, 2-napthyl, 2-furyl, 3-furyl, 2-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl and phenyl having one to three substituents which

are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, CF3, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, O-benzyl, O-phenyl, amino and phenyl;

wherein D is either hydrogen or U; E is either oxygen or CH-U, provided that if D is hydrogen, then E is CH-U, or if E is oxygen then D is U;

wherein U is hydrogen, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or branched alkyl or (C1-C4)-straight or branched alkenyl, 2-indolyl, 3-indolyl, [(C1-C4)-alkyl or (C1-C4)-alkenyl]-Ar or Ar (Ar as described above);

wherein J is hydrogen or C1 or C2 alkyl or benzyl; K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylethyl; or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain an oxygen (O), sulfur (S), SO or SO2 substituted therein.

The stereochemistry at position 1 (Formula I) is (R) or (S), with (S) preferred.

<u>K_i Test Procedure</u>

Inhibition of the peptidyl-prolyl isomerase

(rotamase) activity of the compounds used herein can be evaluated by known methods described in the literature (Harding, M.W. et al. *Nature* 341: 758-760 (1989); Holt et al. *J. Am. Chem. Soc.* 115: 9923-9938). These values are obtained as apparent $_K$'s and are presented for various compounds in Table I. The *cis-trans* isomerization of an alanine-proline bond in a model substrate, N-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide, is monitored spectrophotometrically in a chymotrypsin-coupled assay, well known to those skilled in the art, which releases *para*-nitroanilide from the *trans* form of the substrate. The inhibition of this reaction caused by the addition of different concentrations of inhibitor is determined, and the data is analyzed as a change in first-order rate constant as a function of inhibitor concentration to yield the apparent $_K$ values.

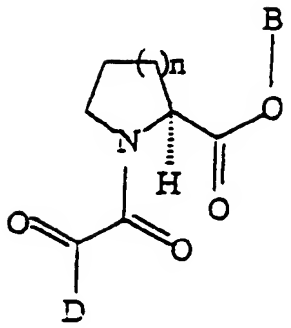
In a plastic cuvette are added 950 mL of ice cold assay buffer (25 mM HEPES, pH 7.8, 100 mM NaCl), 10 mL of FKBP (2.5 mM in 10 mM Tris-Cl pH 7.5, 100 mM NaCl, 1 mM dithiothreitol), 25 mL of chymotrypsin (50 mg/ml in 1 mM HCl) and 10 mL of test compound at various concentrations in dimethyl sulfoxide. The reaction is initiated by the addition of 5 mL of substrate (succinyl-Ala-Phe-Pro-Phe-para-nitroanilide, 5 mg/mL in 2.35 mM LiCl in trifluoroethanol).

The absorbance at 390 nm versus time is

monitored for 90 sec using a spectrophotometer and the rate constants are determined from the absorbance versus time data files.

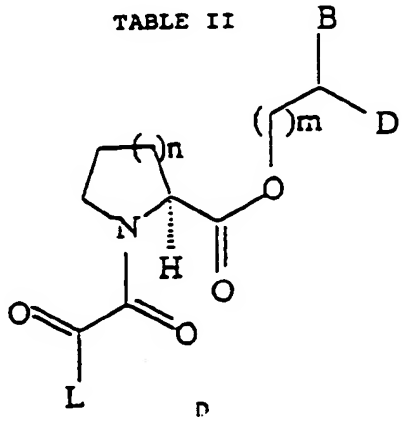
The data for these experiments is presented in Tables I and IV.

TABLE I



No.	B	D	n	K
1	Benzyl	Phenyl	2	1.5 μM
2	3-Phenylpropyl	Phenyl	2	
3	4-(4-Methoxy-phenyl)butyl	Phenyl	2	
4	4-Phenylbutyl	Phenyl	2	0.35 μM
5	Phenethyl	Phenyl	2	1.1 μM
6	4-Cyclohexyl-butyl	Phenyl	2	0.4 μM
7	Benzyl	Methoxy	2	80 μM
8	4-Cyclohexyl-butyl	Methoxy	2	6 μM
9	3-Cyclohexyl-propyl	Methoxy	2	20 μM
10	3-Cyclopentyl-propyl	Methoxy	2	35 μM
11	Benzyl	2-Furyl	2	3 μM
12	4-Cyclohexyl-butyl	3,4,5-Trimethoxy-phenyl	2	0.04 μM
13	3-Phenoxy-benzyl	3,4,5-Trimethoxy-phenyl	2	0.018 μM
14	4-Phenylbutyl	3,4,5-Trimethoxy-phenyl	2	0.019 μM
15	3-(3-Indolyl) propyl	3,4,5-Trimethoxy-phenyl	2	0.017 μM
16	4-(4-Methoxy-phenyl)butyl	3,4,5-Trimethoxy-phenyl	2	0.013 μM

TABLE II



No.	n	m	B	D	L
17	2	0	3-Phenylpropyl	3-(3-Pyridyl) propyl	Phenyl
18	2	0	3-Phenylpropyl	3-(2-Pyridyl) propyl	Phenyl
19	2	0	3-Phenylpropyl	2-(4-Methoxy-phenyl)ethyl	Phenyl
20	2	0	3-Phenylpropyl	3-Phenylpropyl	Phenyl
21	2	0	3-Phenylpropyl	3-Phenylpropyl	3,4,5-Trimeth-oxyphenyl
22	2	0	3-Phenylpropyl	2-(3-Pyridyl)	3,4,5-Trimeth-oxyphenyl
23	2	0	3-Phenylpropyl	3-(2-Pyridyl)	3,4,5-Trimeth-oxyphenyl
24	2	0	3-Phenylpropyl	3-(4-Methoxy-phenyl)propyl	3,4,5-Trimeth-oxyphenyl
25	2	0	3-Phenylpropyl	3-(3-Pyridyl) propyl	3-Iso-propoxy-phenyl

Chick Dorsal Root Ganglion Cultures and Neurite Outgrowth

Dorsal root ganglia were dissected from chick embryos of ten day gestation. Whole ganglion explants were cultured on thin layer Matrigel-coated 12 well plates with Liebovitz L15 plus high glucose media supplemented with 2mM glutamine and 10% fetal calf serum, and also containing 10 μM cytosine β-D arabinofuranoside (Ara C) at 37°C in an environment containing 5% CO_2. Twenty-four hours later, the DRGs were treated with various concentrations of nerve

growth factor, immunophilin ligands or combinations of NFG plus drugs. Forty-eight hours after drug treatment, the ganglia were visualized under phase contrast or Hoffman Modulation contrast with a Zeiss Axiovert inverted microscope. Photomicrographs of the explants were made, and neurite outgrowth was quantitated. Neurites longer than the DRG diameter were counted as positive, with total number of neurites quantitated per each experimental condition. Three to four DRGs are cultured per well, and each treatment was performed in duplicate. Data from 6-10 different concentrations were used to generate dose-response curves, from which ED_{50} values were calculated. Typical response curves are shown in Figures 1 and 2 for examples 15 and 22, respectively.

Data for these experiments are presented in Tables III and IV. Representative photomicrographs of sensory neurons responding to the trophic effects of the drugs are shown in Figures 3-5. Fig. 3 demonstrates the dose-dependent neurotrophic effects of example 21 on neuronal cultures. Figure 4 demonstrates the stimulation of neurite outgrowth induced by a 300 pM dose of example 15 and, Fig. 5 demonstrates the stimulation of neurite outgrowth

induced by a 1nM dose of example 22.

Table III
Neurite Outgrowth in Chick DRG

Example	ED_{50}, nM Neurite Outgrowth in DRG cultures
1	25-100
2	10-20
3	0.500
4	25-100
5	25-100
6	10-20
7	>10,000
8	>10,000
9	>10,000
10	>10,000
11	1000
12	0.031
13	0.180
14	1-5
15	0.055
16	0.030

Table IV

Biological Results

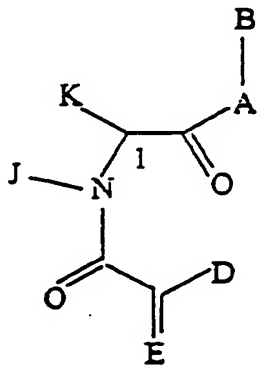
Compound in	K, nM	ED_{50}, nM Neurite Outgrowth DRG cultures
17	56	1-5
18	50	0.063
19	270	10-20
20	---	0.0044
21	1.0	0.61
22	3.0	0.95
23	1.0	25
24	3.0	0.50
25	2.0	0.30

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modifications are intended to be included within the scope of the following claims.

What is claimed is:

1. A method of treating a neurological activity in an animal, comprising:

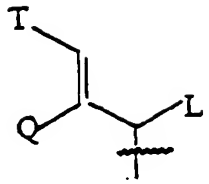
administering to an animal an effective amount of a pipecolic acid derivative represented by the formula



and pharmaceutically acceptable salts thereof,

wherein A is CH_2, O, NH, or N-(C1-C4 alkyl);

wherein B and D are independently Ar, (C5-C7)-cycloalkyl substituted (C1-C6)-straight or branched alkyl or alkenyl, (C5-C7)-cycloalkenyl substituted (C1-C6)-straight or branched alkyl or alkenyl, or Ar substituted (C1-C6)-straight or branched alkyl or alkenyl, wherein in each case, one or two carbon atoms of the straight or branched alkyl or alkenyl groups may be substituted with 1-2 heteroatoms selected from the group consisting of oxygen, sulfur, SO and SO_2 in chemically reasonable substitution patterns, or



wherein Q is hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl;

wherein T is Ar or substituted 5-7 membered cycloalkyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C1-C4)-alkyl or O-(C1-C4)-alkenyl and carbonyl;

wherein Ar is selected from the group consisting of 1-napthyl, 2-napthyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl and phenyl, monocyclic and bicyclic heterocyclic ring systems with individual ring sizes being 5 or 6 which may contain in either or both rings a total of 1-4 heteroatoms independently selected from oxygen, nitrogen and sulfur; wherein Ar may contain one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, hydroxymethyl, nitro, CF3, trifluoromethoxy, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, O-(C1-C4)-

straight or branched alkyl or 0-(C1-C4)-straight or branched alkenyl, 0-benzyl, 0-phenyl, amino, 1, 2-methylenedioxy, carbonyl and phenyl;

wherein L is either hydrogen or U; M is either oxygen or CH-U, provided that if L is hydrogen, then M is CH-U, or if M is oxygen then L is U;

wherein U is hydrogen, 0-(C1-C4)-straight or branched alkyl or 0-(C1-C4)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or branched alkyl or (C1-C4)-straight or branched alkenyl [(C1-C4)-alkyl or (C1-C4)-alkenyl]-Ar or Ar (Ar as described above);

wherein J is hydrogen or C1 or C2 alkyl or benzyl; K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylmethyl; or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain an oxygen (O), sulfur (S), SO or SO_2

substituted therein;

wherein n is 0-3; and

wherein said pipecolic acid derivative has an affinity for FKBP-type immunophilins, said administering stimulates growth of damaged peripheral nerves or promotes neuronal regeneration, the FKBP-type immunophilin exhibits rotamase activity, and the pipecolic acid derivative

inhibits said rotamase activity of the immunophilin.

2. The method of claim 1, wherein the neuronal activity is selected from the group consisting of stimulation of damaged neurons, promotion of neuronal regeneration, prevention of neurodegeneration and treatment of neurological disorders.

3. The method of claim 2, wherein the neurological disorder is selected from the group consisting of peripheral neuropathies cause by physical injury or disease state, physical damage to the brain, physical damage to the spinal cord, stroke associated with brain damage, and neurological disorders relating to neurodegeneration.

4. The method of claim 3, wherein the neurological disorder is selected from the group consisting of Alzheimer's Disease and Parkinson's Disease.

5. The method of claim 1, wherein the pipecolic acid derivative compound is immunosuppressive or non-immunosuppressive.

6. A method of treating a neurological activity in an animal, comprising:

administering to an animal an effective amount of a pipecolic acid derivative according to claim 1 having an affinity for FKBP-type immunophilins in combination with an effective amount of a neurotrophic factor selected from the group consisting of neurotrophic growth factor, brain derived growth factor, glial derived growth factor, ciliai neurotrophic factor, and neurotropin-3, to stimulate growth of damaged peripheral nerves or to promote neuronal regeneration, wherein the FKBP-type immunophilin exhibits rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

7. The method of claim 6, wherein the neuronal activity is selected from the group consisting of stimulation of damaged neurons, promotion of neuronal regeneration, prevention of neurodegeneration and treatment of neurological disorders.

8. The method of claim 7, wherein the neurological disorder is selected from the group consisting of peripheral neuropathies caused by physical injury or disease state, physical damage to the brain, physical damage to the spinal cord, and neurological disorders relating to neurodegeneration.

9. The method of claim 6, wherein the neurological disorder is selected from the group consisting of Alzheimer's Disease and Parkinson's Disease.

10. The method of claim 6, wherein the pipecolic acid derivative compound is immunosuppressive or non-immunosuppressive.

11. A method of stimulating growth of damaged peripheral nerves, comprising;

administering to damaged peripheral nerves an effective amount of a pipecolic acid derivative compound according to claim 1 having an affinity for FKBP-type immunophilins to stimulate or promote growth of the damaged peripheral nerves, wherein the FKBP-type immunophilins exhibit rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

12. The method of claim 11, further comprising administering a neurotrophic factor to stimulate or promote growth of the damaged peripheral nerves selected from the group consisting of neurotrophic growth factor, brain derived growth factor, glial derived growth factor, cilial neurotrophic factor, and neurotropin-3.

13. The method of claim 11, wherein the pipecolic acid derivative is immunosuppressive or non-immunosuppressive.

14. A method for promoting neuronal regeneration and growth in animals, comprising:

administering to an animal an effective amount of a pipecolic acid derivative compound according to claim 1 having an affinity for FKBP-type immunophilins to promote neuronal regeneration, wherein the FKBP-type immunophilins exhibit rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

15. The method of claim 14, further comprising administering an effective amount of a neurotrophic factor to promote neuronal regeneration selected from the group consisting of neurotrophic growth factor, brain derived growth factor, glial derived growth factor, and neurotropin-3.

16. The method of claim 14, wherein the pipecolic acid derivative compound is immunosuppressive or non-immunosuppressive.

17. A method for preventing neurodegeneration in an animal, comprising:

administering to an animal an effective amount of a pipecolic acid derivative according to claim 1 having an affinity for FKBP-type immunophilins to prevent neurodegeneration, wherein the FKBP-type immunophilin exhibits rotamase activity and the pipecolic acid derivative inhibits said rotamase activity of the immunophilin.

18. The method of claim 17, further comprising administering an effective amount of a neurotrophic factor to prevent neurodegeneration selected from the group consisting of neurotropic growth factor, brain derived growth factor, glial derived growth factor, cilial neurotropic factor, and neurotropin-3.

19. The method of claim 17, wherein the pipecolic acid derivative compound is immunosuppressive or non-immunosuppressive.

20. The method of treating a neurological activity according to claim 1 represented by the formula:

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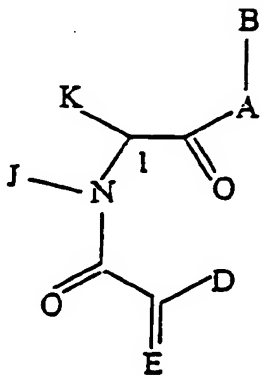
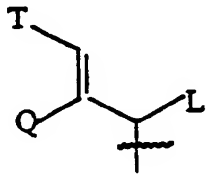


and pharmaceutically acceptable salts thereof,

wherein A is O, NH, or N-(C1-C4 alkyl);

wherein B is hydrogen, CHL-Ar, (C1-C6)-straight or branched alkyl, (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl or Ar substituted (C1-C6)-alkyl or alkenyl, or



wherein L and Q are independently hydrogen, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl;

wherein T is Ar or substituted cyclohexyl with substituents at positions 3 and 4 which are independently selected from the group consisting of hydrogen, hydroxyl, O-(C1-C4)-alkyl or O-(C1-C4)-alkenyl and carbonyl;

wherein Ar is selected from the group consisting of 1-napthyl, 2-napthyl, 2-furyl, 3-

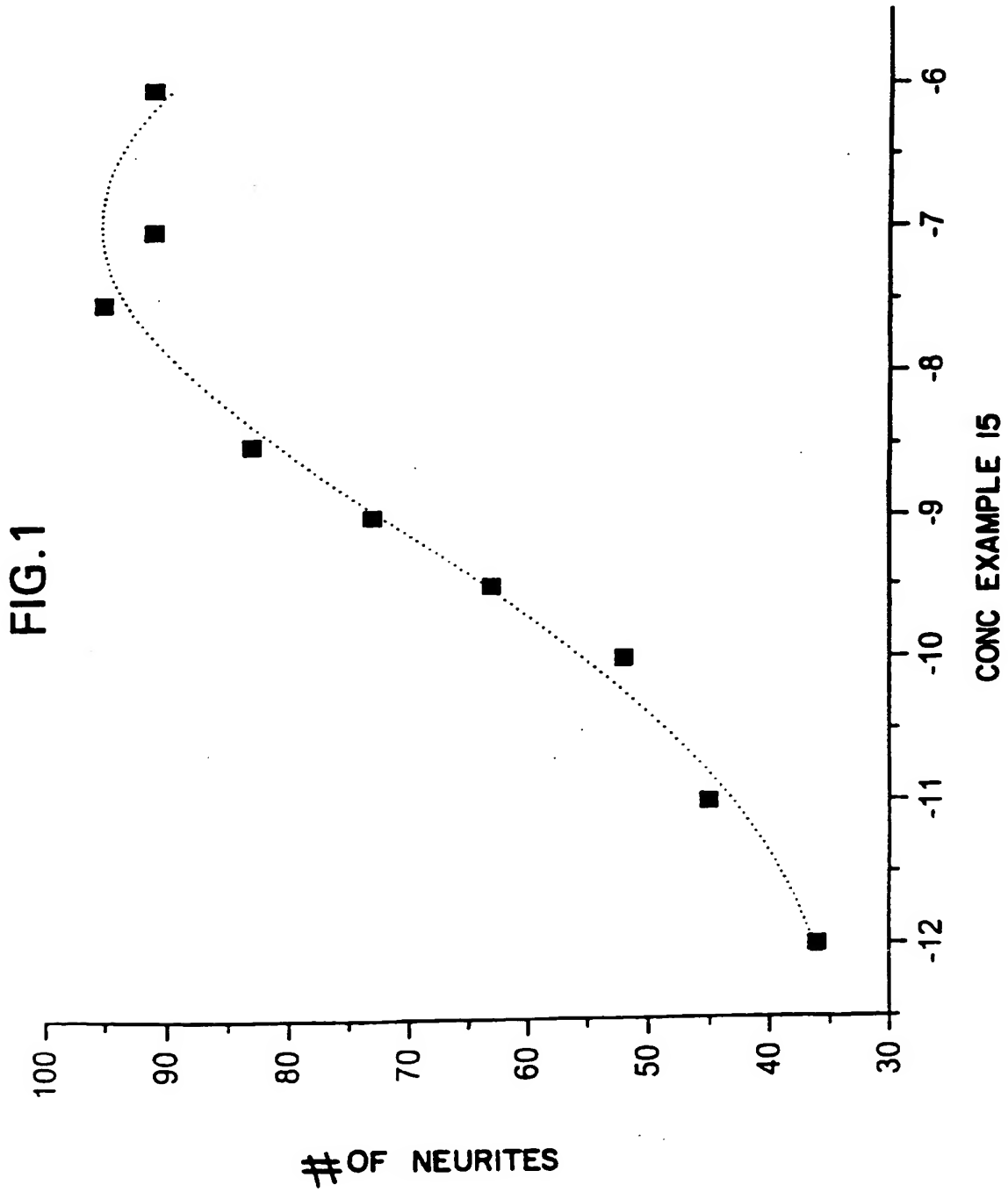
furyl, 2-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl and phenyl having one to three substituents which are independently selected from the group consisting of hydrogen, halo, hydroxyl, nitro, CF3, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, O-benzyl, O-phenyl, amino and phenyl.

wherein D is either hydrogen or U; E is either oxygen or CH-U, provided that if D is hydrogen, then E is CH-U, or if E is oxygen then D is U;

wherein U is hydrogen, O-(C1-C4)-straight or branched alkyl or O-(C1-C4)-straight or branched alkenyl, (C1-C6)-straight or branched alkyl or (C1-C6)-straight or branched alkenyl, (C5-C7)-cycloalkyl, (C5-C7)-cycloalkenyl substituted with (C1-C4)-straight or branched alkyl or (C1-C4)-straight or branched alkenyl, 2-indolyl, 3-indolyl, [(C1-C4)-alkyl or (C1-C4)1alkenyl]-Ar or Ar (Ar as described above);

wherein J is hydrogen or C1 or C2 alkyl or benzyl; K is (C1-C4)-straight or branched alkyl, benzyl or cyclohexylethyl; or wherein J and K may be taken together to form a 5-7 membered heterocyclic ring which may contain an oxygen (O), sulfur (S), SO or SO2 substituted therein.

FIG.1


-6
-7
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CONC EXAMPLE 15
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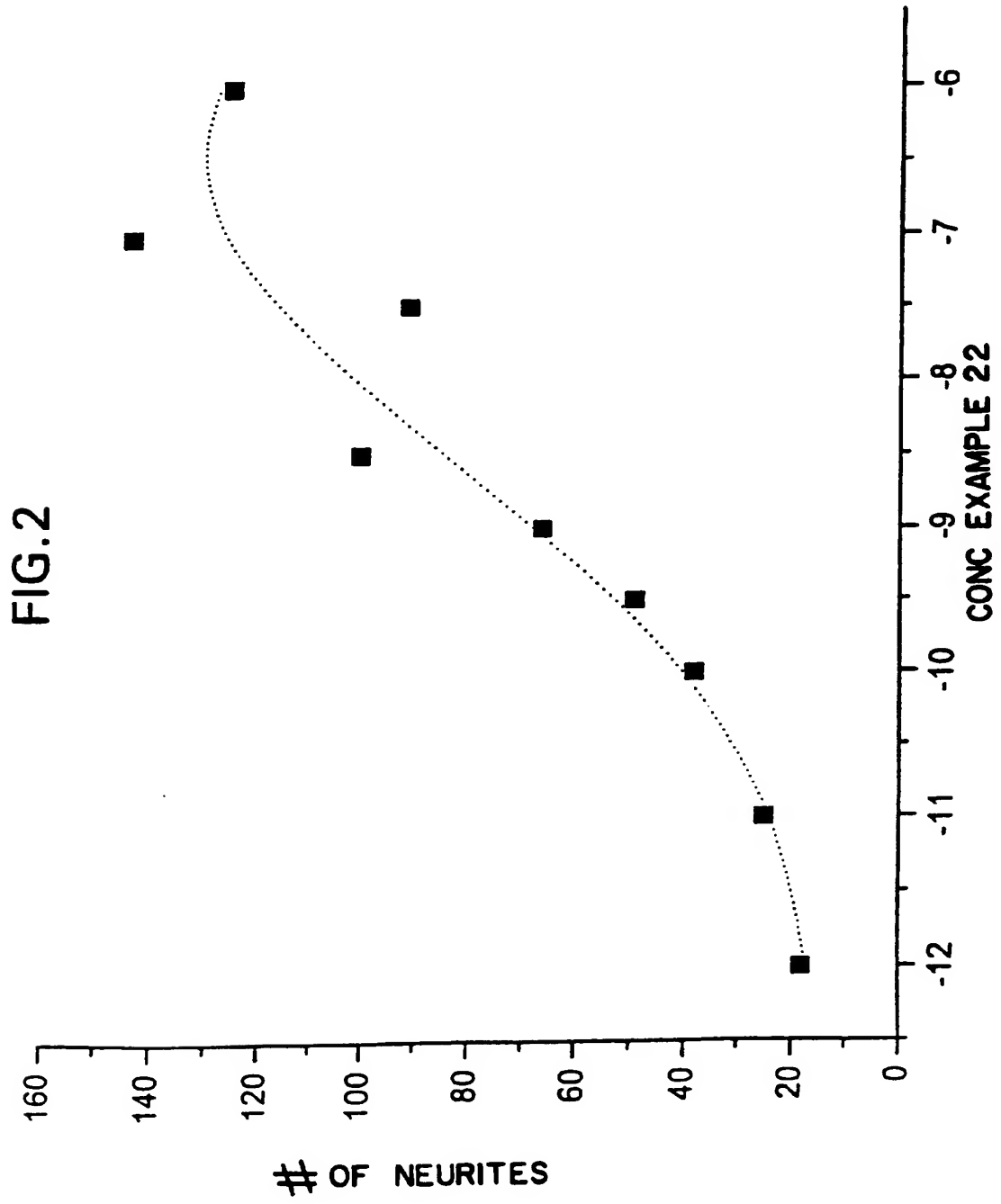
90
80
70
60
50
40
30
OF NEURITES

FIG.2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CONC EXAMPLE 22
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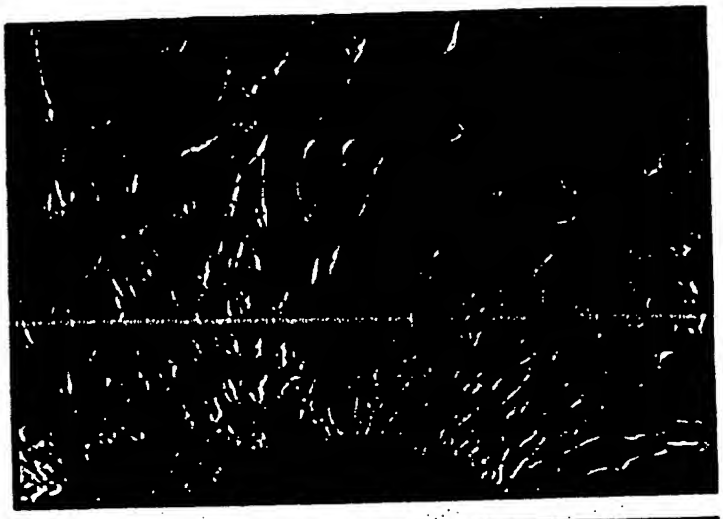

FIG.3C

FIG.3B

FIG.3A

FIG.4

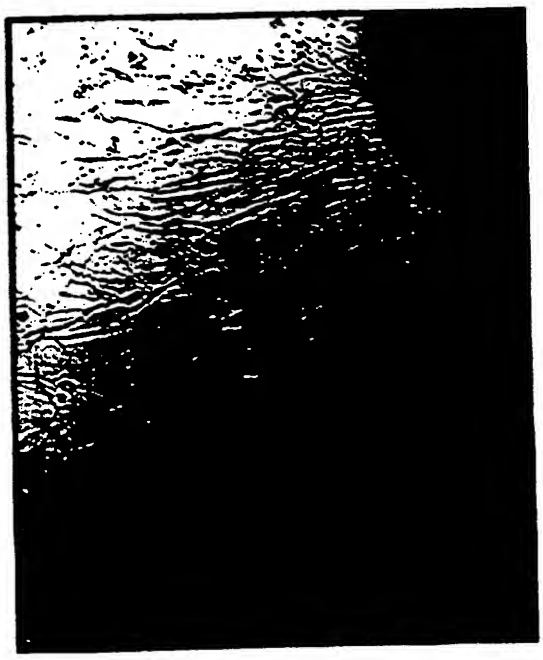

FIG.5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/13624

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/445, 31/40
US CL :514/330, 423, 428, 885

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/330, 423, 428, 885

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,330,993 A (ARMISTEAD ET AL.) 19 July 1994, column 4, line 10.	1-20
A	US 4,535,167 A (FREIDINGER) 13 August 1985, column 6, Table 1.	1-20
Y	US 5,192,773 A (ARMISTEAD ET AL.) 09 March 1993, column 3, line 25.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 NOVEMBER 1996	Date of mailing of the international search report 04 DEC 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KEITH MACMILLAN Telephone No. (703) 308-1235

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